Attorney Docket No. 71417/49138

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Kearney et al.

U.S.S.N: 09/961,128

FILED: September 21, 2001

FOR: ENDOTHELIAL CELL MITOGEN BIOASSAY

Art Unit: 1636

Examiner: Celine Qian

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" service under 37 CFR 1.10 in an envelope addressed to: Mail Stop: AF ~~Amendment~~, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 and designated by the "Express Mail" mailing label No. EV 492343022 US on June 28, 2005.

By Maggie Hamelin

Mail Stop: Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MARIANNE KEARNEY PURSUANT TO 37 CFR 1.132

I, Marianne Kearney, declare as follows:

1. I am an inventor of the present invention, which is the subject of the above-identified application. Since 2003, I have been a Cardiovascular Regulatory Liaison/Project Manager at Caritas St. Elizabeth's Medical Center of Boston, Inc. ("Caritas"), which holds the rights to the above-identified application. I am also a member of the Institutional Biosafety Committee (IBC). Prior to accepting my current position, I had previously been a Cardiovascular Research Assistant/Laboratory Supervisor for thirteen years, during which time I also served as a Coordinator for two multi-center randomized clinical trials at the Core Pathology Laboratory, where I trained and supervised site coordinators at over fifty

(50) participating hospitals. I also designed and coordinated pre-clinical studies for IND submissions to the Food & Drug Administration (FDA). I was responsible for setting up a Human Gene Therapy Laboratory, where I prepared plasmid DNA for administration to patients enrolled in FDA approved gene therapy trials. I was also responsible for setting up a Human Cellular Therapy Laboratory utilized for preparation of autologous cellular products for administration to patients enrolled in FDA-approved cellular therapy trials. I hold a Bachelor of Arts degree in Biology from Rhode Island College, Rhode Island (1990), and I hold a Certificate in Clinical Research from Boston University, Boston, Massachusetts (2003). I am a member of the Association of Clinical Research Professionals (ACRP), the International Society of Cellular Therapy (ISCT), and the Regulatory Affairs Professionals (RAPS). I co-authored a book (Isner & Kearney, Atherosclerosis: Pathology of the Vasculature in Live Patients, Harcourt Brace & Co., London, UK (1999)) with the now-deceased Dr. Jeffrey Isner, the co-inventor of the present invention. I have also co-authored four (4) book chapters and over sixty (60) scientific papers, and I have prepared and delivered scientific presentations.

2. The subject application discloses among other things and claims a method for testing a test plasmid containing a gene encoding for an endothelial cell mitogen for the ability to produce a biologically active endothelial cell mitogen protein, wherein endothelial cells demonstrate enhanced survival in a cell survival assay in response to conditioned media from a transfection host cell line transfected with the test plasmid in comparison to conditioned media from a transfection host cell line transfected with a control plasmid, the method comprising (a) transiently transfecting a transfection host cell line with a test plasmid containing a gene encoding for an endothelial cell mitogen; (b) incubating test sample endothelial cells with conditioned media from the transiently transfected transfection host cell line; (c) determining the ability of the test sample endothelial cells to reduce MTS to formazan; and (d) determining the level of cell survival of the test sample endothelial cells incubated with conditioned media from the transfection host cell line transfected with

the test plasmid containing a gene encoding for an endothelial cell mitogen as compared to control sample endothelial cells incubated with conditioned media from the transfection host cell line transfected with a control plasmid; wherein the level of cell survival of both the test sample and control sample endothelial cells is determined by the ability of the endothelial cells to reduce MTS to formazan; and wherein the level of cell survival of the test sample endothelial cells indicates that the test plasmid produces a biologically active endothelial cell mitogen.

3. The subject application also discloses among other things and claims a method for evaluating the ability of a first plasmid DNA construct containing a gene encoding for an endothelial cell mitogen to produce a bioactive endothelial cell mitogen protein as compared to the ability of a second plasmid DNA construct containing a gene encoding for an endothelial cell mitogen to produce a bioactive endothelial cell mitogen protein, wherein endothelial cells demonstrate enhanced survival in a cell survival assay in response to conditioned media from a transfection host cell line transfected with the first plasmid in comparison to a transfection host cell line transfected with the second plasmid, the method comprising (a) transiently transfecting a first sample of a transfection host cell line with the first plasmid DNA construct containing a gene encoding for an endothelial cell mitogen; (b) incubating endothelial cells with conditioned media from the transiently transfected transfection host cell line of step a; (c) transiently transfecting a second sample of the transfection host cell line with the second plasmid DNA construct containing a gene encoding for an endothelial cell mitogen; (d) incubating endothelial cells with conditioned media from the transiently transfected transfection host cell line of step c; (e) determining the ability of the endothelial cells transfected with the first plasmid to reduce MTS to formazan in comparison with the ability of the endothelial cells transfected with the second plasmid to reduce MTS to formazan; and (f) determining the level of cell survival of the endothelial cells of step b incubated with conditioned media from the transfection host cell line transfected with the first plasmid containing a gene encoding for an endothelial cell

mitogen as compared to the endothelial cells of step d incubated with conditioned media from the transfection host cell line transfected with the second plasmid containing a gene encoding for an endothelial cell mitogen; wherein the level of cell survival of the endothelial cells is determined by the ability of the endothelial cells incubated with conditioned media from the first sample transfected with the first plasmid to reduce MTS to formazan in comparison with the ability of the endothelial cells incubated with conditioned media from the second sample transfected with the second plasmid to reduce MTS to formazan and wherein the levels of cell survival of the endothelial cells indicate that the first plasmid produces an active endothelial cell mitogen.

4. Traditionally, a cell may be stably or transiently transfected. A transfected cell is a cell into which cloned genetic material, usually an infectious viral or other plasmid or cosmid DNA, has been introduced by infection. Exposed cells take up the foreign DNA and transport it to the nucleus, where it can be transcribed. In this instance, the cells are transiently transfected. This method of transfecting cells results in cells that are typically mixed populations of cells, including some cells that are not transfected. In contrast, stable transfection techniques select only for the transfected cells.. As a result, the foreign DNA is passed to the progeny cells during cell division, resulting in a stably transfected cell line. See, e.g., Cooper, G.M., The Cell: A Molecular Approach, (ASM Press (Washington, D.C.)/Sinauer Associates (Sunderland, MA), 1997), p. 122. Although the cells usually produce a higher level of foreign protein, transient transfection is used in this case, to mimic the anticipated in vivo effect of transfection

5. The current invention addresses these concerns and many other issues as well. The present invention provides, among other things, methods of rapidly and efficiently testing and evaluating DNA plasmids utilizing transient cell transfection to produce the foreign protein.

6. Traditionally, there is a distinction between cell proliferation and cell viability. One example of a cell mitogenic assay is that of using incorporation of ^{3}H-thymidine during the cell cycle as a means of measuring cell proliferation. Alternatively, one example of a cell viability assay is to measure cell survival by tetrazolium (MTS)/formazan assay.

7. The current invention utilizes analysis of cell viability as a tool to confirm sufficient protein production by the gene construct , because the proposed gene product (VEGF proteins) is known to act as a survival factor (increasing cell viability) for specific cell types such as Human Umbillical Vein Endothelial Cells.

8. I have reviewed the Patent Office Action ("Office Action") dated December 28, 2004, issued in connection with the subject application. As I understand the Office Action, the Patent Examiner has rejected certain claims of the application in view of documents that include the following: Sugihara et al. (J. Biol. Chem. 273 (5): 3033-3038 (1998) ("Sugihara")); Buttke et al. (J. Immunol. Meth. 157: 233-240 (1993) ("Buttke")); and Breier et al. (Devel. 114: 521-532 (1992) ("Breier")).

9. I disagree with these claim rejections.

10. The current invention illustrates that media obtained from transient cell transfection is sufficient to be used in an assay for testing and evaluating a plasmid DNA construct for its ability to produce a bioactive endothelial cell mitogen protein as determined by the ability of the endothelial cells to reduce MTS to formazan as an indicator of cell survival. The specification of the current patent application describes examples of comparing a test plasmid with a control and also comparing multiple plasmids.

11. This result is completely unexpected.

12. For example, Sugihara describes a cell mitogenic assay using incorporation of ^{3}H-thymidine during the cell cycle as a means of measuring cell proliferation. In contrast, the present invention provides a method for testing the survival of cells, that is, their ability to overcome cell death, as measured by a cell viability assay. These traits are quite different, because viable cells are not necessarily undergoing mitosis. Sugihara does not teach or suggest measuring of cell survival at all and does not teach or suggest the desirability of doing so.

13. For example, Buttke teaches an MTS/formazan assay. Buttke repeatedly distinguishes between the use of the ^{3}H-thymidine assay to measure cell proliferation and the use of the MTS/formazan assay to measure cell viability. Moreover, Buttke repeatedly emphasizes the use of both assays to distinguish between cell proliferation and cell viability using the FDC-P1 cell line. While Buttke provides instances in which cell proliferation and viability coincide, Buttke also provides an example in which cell proliferation ceases while cell viability continues.

14. For example, Breier teaches that conditioned media following transient transfection of expression vector comprising VEGF cDNA to Cos-1 cells can be collected and assayed for mitogenic activity on bovine aortic endothelial cells, but this is not a description of cell survival. Breier equates "mitogenic effect" with stimulating the proliferation of endothelial cells. Breier's assay is merely the counting of cells in a Coulter counter to measure proliferation of endothelial cells – not the MTS/formazan assay of the present invention.

15. Additionally, these references would not have been combined by those of ordinary skill in the art.

16. The cell proliferation assay of Sugihara and the cell viability assay of Buttke are measuring two different parameters. There is no motivation for one of skill in the art to use the stable transfection technique and cell proliferation assay of Sugihara to perform the transient transfection and cell viability assay of the present invention. The cell proliferation assay described in Sugihara does not necessarily provide a measurement of cell viability as provided according to the present invention or as provided in Buttke. Moreover, there is no suggestion in Sugihara that measurement of cell viability, as opposed to cell proliferation, would be desirable. Likewise, there is no suggestion in Buttke that measurement of cell proliferation would necessarily be interchangeable with measurement of cell viability. While Buttke provides instances in which cell proliferation and viability coincide, Buttke also provides an example, described above, in which cell proliferation ceases while cell viability continues. Therefore, Buttke teaches away from Sugihara by emphasizing a marked preference for the need for both tests as a means of measuring and comparing two different cell parameters, including an example in which the results of the two assays differed precisely because they were measuring two different parameters. As a result, any technical advantages of the MTS/formazan assay over the ^{3}H-thymidine assay are essentially irrelevant, because the assays are measuring two different cell parameters.

17. Similar to Sugihara, Breier describes a cell mitogenic assay using a Coulter counter to count cells as a means of measuring cell proliferation. In contrast, the present invention provides a method for testing the survival of cells, that is, their ability to overcome cell death, as measured by a cell viability assay. Breier never distinguishes between cells at different stages of mitosis, but simply counts the number of cells, which cannot distinguish between the two parameters of cell viability and cell proliferation, whereas Buttke uses the ^{3}H-thymidine assay, rather than the Coulter counter, to measure cell proliferation. Similar to Sugihara, therefore, Buttke teaches away from Breier by emphasizing a marked preference for the need for measuring and comparing two different cell parameters. The use of COS-1 cells in the Breier assay is irrelevant, because the parameters being measured are different.

18. In relation to the present invention, therefore, the present invention is completely unexpected.

19. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, and that such willful false statements may jeopardize the validity of the application on any patent issued thereon.

Date: June 28, 2005

Marianne Kearney